EXHIBIT 2

New Drug Development

RECOMBINANT HUMAN TUMOR NECROSIS FACTOR RECEPTOR (p75) Fc FUSION PROTEIN (TNFR:Fc) IN RHEUMATOID ARTHRITIS

Kim M Murray and Stephen L Dahl

BACKGROUND: Tumor necrosis factor (TNF) is the dominant mediator of the cytokine cascade that causes inflammation and joint destruction in rheumatoid arthritis. A new class of agents under investigation, the biologic TNF inhibitors, inhibits the activity of TNF. Recombinant human TNF receptor p75 Fc fusion protein (TNFR:Fc; Enbrel) blocks the activity of the cytokine TNF. The preclinical, Phase I, and Phase II data of TNFR:Fc in rheumatoid arthritis are reviewed in this article.

METHODS: All available data on TNFR:Fc in rheumatoid arthritis were reviewed. These data included published literature and data on file at the manufacturer.

RESULTS: TNFR:Fc has been effective in many models of inflammation, including animal models of rheumatoid arthritis and in clinical rheumatoid arthritis trials. Conclusions from a study with TNFR "knockout" mice (genetically altered mice incapable of producing TNFR proteins) demonstrated that p75 TNFR is a natural antagonist of TNF-mediated inflammation. A placebo-controlled, dose-escalation, Phase I trial evaluated the safety and efficacy of TNFR:Fc in patients with rheumatoid arthritis. There were no serious adverse effects reported. A Phase II, randomized, double-blind, placebo-controlled trial evaluated 180 patients with active rheumatoid arthritis whose previous therapy had failed. A dose–response relationship was observed in the number of tender and swollen joints; patients who received the highest dose (16 mg/m²) of TNFR:Fc had the greatest improvement. Treatment was generally well tolerated. TNFR:Fc is nonimmunogenic; no antibodies to TNFR:Fc have been detected thus far in human studies.

CONCLUSIONS: Preliminary data indicate that TNFR:Fc is an excellent candidate for future long-term studies in the treatment of rheumatoid arthritis.

KEY WORDS: tumor necrosis factor, rheumatoid arthritis, recombinant human tumor necrosis factor receptor p75 Fc fusion protein.

Ann Pharmacother 1997;31:1335-8.

RHEUMATOID ARTHRITIS is a chronic, progressive, systemic, inflammatory disorder of unknown etiology characterized by symmetric, erosive, disabling, deforming polyarthritis and a wide array of extraarticular complications.[1] There is no known cure for rheumatoid arthritis, but treatment with nonsteroidal antiinflammatory drugs (NSAIDs) or glucocorticoids may provide relatively rapid attenuation of joint pain and swelling, reduce duration and intensity of morning stiffness, and reduce fatigue.[2] Disease-modifying antirheumatic drugs (DMARDs) may be added to NSAIDs and/or glucocorticoids and may delay disease progression. These agents include hydroxychloroquine, methotrexate, sulfasalazine, D-penicillamine, gold (chrysotherapy), and azathioprine. Cyclosporine is another option for patients refractory to other therapeutic interventions.[3] These agents have adverse event profiles that may limit their long-term administration.[4,5] To improve response rates, both in terms of proportion of responders and degree of response, combination DMARD therapy is becoming commonplace.[6-8]

Despite the increasing number of treatment options, many patients remain unresponsive to, become unresponsive to, or cannot tolerate available treatments. Consequently, research continues for new and better-tolerated therapies to attenuate the inflammation and pain associated with rheumatoid arthritis, and to halt progression of erosive joint damage. A new class of agents under investigation, the biologic tumor necrosis factor (TNF) inhibitors, includes recombinant TNF receptors and anti-TNF monoclonal antibodies. These agents inhibit TNF, a specific activator of the immune system. Recombinant human TNF receptor p75 Fc fusion protein (TNFR:Fc; Enbrel, Immunex, Seattle, WA), which blocks the activity of the cytokine TNF, is reviewed in this article.

Biologic Activities of Tumor Necrosis Factor

TNF is one of the most abundant cytokines produced by macrophages and activated T cells, especially endotoxin-stimulated macrophage. TNF exists as a transmembrane protein and can circulate in the serum as soluble TNF after being cleaved from the cell surface by proteolysis. It was named TNF because it was found in the serum of animals receiving endotoxin and could induce hemorrhagic necrosis in tumors.[9]

At low concentrations, TNF enhances the protective inflammatory response. It aids in extravasation of neutrophils, lymphocytes, and monocytes into the tissues by in-

Kim M Murray PharmD, Professional Services Manager, Immunex Corporation, Seattle, WA; Clinical Assistant Professor, Department of Pharmacy, University of Washington, Seattle
Stephen L Dahl PharmD BCFS, Associate Professor of Medicine, School of Medicine, University of Missouri–Kansas City, Kansas City, MO
Reprints: Kim M Murray PharmD, Immunex Corporation, Professional Services Department, 51 University St., Seattle WA 98101, FAX 800/221-6920

creasing the expression of adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, endothelial leukocyte adhesion molecule-1) on vascular endothelial cells.[10] TNF activates and enhances the function of various leukocytes, including neutrophils, macrophages, and eosinophils. At high concentrations, TNF can function as a pyrogen by acting directly on the hypothalamus to elevate the body's thermal set point. In addition, it can activate the clotting system and induce production of acute-phase proteins, and can cause suppression of the bone marrow.

TNF mediates the cytokine cascade that causes inflammation and joint destruction in rheumatoid arthritis. TNF concentrations are elevated by up to 50% in rheumatoid synovial fluids, particularly in patients with more severe disease or with high concentrations of white blood cells in the synovial fluid.[11] TNF is abundant in macrophages in the rheumatoid synovial membrane and TNF-containing cells are localized to the pannus (articular cartilage in the joints of patients with rheumatoid arthritis), suggesting production near the site of tissue destruction.[12] Furthermore, TNF receptors are localized to macrophages and fibroblasts in the rheumatoid synovial lining layer, as well as to lymphocytes and endothelial cells in the subsynovial membrane, which suggests that a variety of cells in the rheumatoid synovial membrane are potential targets for TNF.[13]

There is increasing evidence that an imbalance among cytokines contributes to the chronic inflammation of rheumatoid arthritis. Interleukin (IL)-2, IL-3, and interferon-gamma are expressed in unexpectedly low concentrations, whereas cytokines generated by macrophages (IL-1, IL-6, TNF, granulocyte-macrophage colony-stimulating factor) are abundantly expressed. Although multiple cytokines are involved in the pathogenesis of rheumatoid arthritis, TNF is the dominant driver of other cytokines, including IL-1 and IL-6.[14]

Naturally Occurring Tumor Necrosis Factor Receptors

Two distinct TNF receptors (TNFRs) have been identified: the 75-kilodalton (kDa) or p75 receptor and the 55-kDa or p55 receptor (formerly referred to as p80 and p60, respectively).[15] Both the p75 and p55 TNFRs exist as cell-surface and soluble forms and both forms bind TNF with equal affinity. TNF cell surface receptors are present on virtually all cell types, including macrophages, lymphocytes, and neutrophils. TNF must bind to two or three cell surface receptor molecules for signaling to occur, resulting in a biologic effect. Binding to a single cell surface receptor does not result in signaling.

Monomeric fragments that comprise the extracellular portion of the cell surface receptors can leave the cell surface and then are referred to as soluble TNFRs (sTNFRs). sTNFRs are present in a number of body compartments and elevated concentrations of sTNFRs have been found in the circulation of patients with rheumatoid arthritis.[16] sTNFR concentrations measured by enzyme-linked immunosorbent assay are higher in synovial fluid samples compared with serum sample concentrations in patients with rheumatoid arthritis. The balance or imbalance between cytokines and their natural inhibitors may play a role in chronic rheumatoid arthritis.[17]

Viruses have used sTNFRs effectively to avoid the human immune response against their invasion. Certain pox viruses have captured the DNA sequence for p75 receptor and used the sequence to encode a protein that neutralizes TNF. This activity may allow the virus to escape the antiviral effects of TNF. These data strongly support the theory that p75 sTNFR is the natural antagonist of TNF activity.[18]

Recombinant Tumor Necrosis Factor Receptor p75 Fusion Protein

Ideal features for a therapeutic entity to treat rheumatoid arthritis include long-term efficacy (symptom relief), convenience of use, and minimal adverse effects. TNFR:Fc, a biologic TNF inhibitor, is a recombinant form of the human p75 sTNFR fused to the Fc fragment of human immunoglobulin G1.[19] The resulting molecule, TNFR:Fc, is a dimer consisting of two sTNFR molecules per Fc molecule expressed in mammalian cells. TNFR:Fc exhibits higher binding affinity for TNF and more potent TNF inhibitory activity in vitro and in a mouse model of septic shock than does the soluble monomeric form of TNF receptor. TNFR:Fc binds TNF with the same high affinity as surface-bound receptors. TNFR:Fc is a potent antagonist of TNF biologic activity both in vitro and in vivo. TNFR:Fc has been effective in many models of inflammation, including animal models of rheumatoid arthritis and in clinical rheumatoid arthritis trials. In addition, the serum half-life of the TNFR:Fc fusion protein is increased dramatically in comparison with the monomer.[20] This longer half-life allows for a convenient dosing schedule. The TNFR:Fc molecule was constructed to provide these features.

Preclinical Trials

Conclusions from a study with TNFR "knockout" mice (mice genetically altered to be incapable of producing TNFR proteins) demonstrated that p75 TNFR is a natural antagonist of TNF-mediated inflammation, while p55 TNFR is an important signaling receptor for inflammatory responses. In animal models of inflammation, TNF is still present in serum, but is biologically inactive after administration of TNFR:Fc. An sTNFR can act as a physical carrier of TNF, but is at the same time an effective TNF antagonist.[18]

In mice with collagen-induced arthritis, TNFR:Fc given on days 14–28 after collagen administration minimized the development of arthritis.[21] The incidence of disease in the treatment group was decreased to 28% compared with 86% in the control group ($p < 0.03$), and the severity of arthritis was also significantly reduced. In mice with established collagen-induced arthritis, TNFR:Fc reduced the severity of disease when given on days 1–14 after disease onset. Disease was less severe in mice treated with TNFR:Fc than in the control group ($p < 0.05$) at 7.5 weeks after disease onset; this difference was even greater at the completion of the experiment at 10 weeks ($p < 0.005$).

Clinical Trials

PHASE I

A placebo-controlled, dose-escalation, Phase I trial evaluated the safety and efficacy of TNFR:Fc in patients with rheumatoid arthritis.[22] Doses of TNFR:Fc 2, 4, 8, or 16 mg/m² administered subcutaneously twice weekly for 4 weeks (8 subcutaneous injections) followed a single intravenous loading dose. At the end of the treatment period, the placebo-treated patients were treated with TNFR:Fc. An additional 6 patients received TNFR:Fc in an open-label phase to obtain additional safety data, for a total of 22 patients treated with TNFR:Fc in this study. There were no serious adverse effects reported. The most common adverse event was mild injection site reactions, which did not cause discontinuation of the drug. All patients tested negative for antibodies to TNFR:Fc. This study had a small number of patients and was designed to determine a safe dose.

In a study of subcutaneous bioavailability of TNFR:Fc, two single 10-mg doses of TNFR:Fc, one subcutaneous and one intravenous, were administered to six (3 women, 3 men) healthy volunteers.[18] Following subcutaneous administration, the mean maximum concentration (C_{max}) was 0.43 ± 0.21 µg/mL, the mean time to reach C_{max} was 66 ± 22 hours, AUC was 81.7 ± 24.6 µg•h/mL, and the apparent clearance was 0.13 ± 0.04 mL/h. The terminal half-life was 92 ± 8 hours and subcutaneous bioavailability was approximately 60%; these parameters provide the rationale for twice-daily subcutaneous dosing. The pharmacokinetic characteristics of TNFR:Fc have not been determined in patients with rheumatoid arthritis.

PHASE II

A Phase II, randomized, double-blind, placebo-controlled trial included 180 patients with active rheumatoid arthritis whose therapy had failed with at least one, but not more than four, DMARDs.[23,24] Patients had at least a 1-month washout of any current DMARD therapy; 33% of patients were receiving methotrexate at the time of screening. Active rheumatoid arthritis was defined as 10 or more swollen joints and 12 or more tender joints. At least one of the following parameters was required for entry into the study: erythrocyte sedimentation rate (ESR) 28 mm/h or more, C-reactive protein (CRP) greater than 2 mg/dL, or morning stiffness lasting 45 minutes or more. At the time of screening, approximately 70% of patients were receiving prednisone dosages of 10 mg/d or more (or its equivalent) and approximately 80% were taking NSAIDs.

Patients were randomized to receive 0.25, 2, or 16 mg/m² of TNFR:Fc or placebo administered subcutaneously twice weekly for 12 weeks. A dose–response relationship was observed in the number of tender and swollen joints; patients who received the highest dose (16 mg/m²) of TNFR:Fc had the greatest improvement. The placebo group and the group that received the lowest TNFR:Fc dose (0.25 mg/m²) exhibited an initial placebo response, but no further improvement was noted thereafter. Patients who received TNFR:Fc 16 mg/m² experienced a mean reduction of 61% ($p < 0.001$) in total swollen joint count compared with those receiving placebo.[23,24]

Patients were also evaluated to determine whether they achieved either 20% or 50% improvement, using the preliminary core set of disease activity measures for rheumatoid arthritis clinical trials proposed by the American College of Rheumatology for active rheumatoid arthritis.[25] Response criteria included a 20% or 50% reduction in the number of swollen and tender joints and the same degree of improvement in at least three of five other variables: visual analog score (VAS) measuring pain, degree of disability Health Assessment Questionnaire (HAQ), patient's global assessment, physician's global assessment, and ESR or CRP. At 3 months 75% of the patients receiving TNFR:Fc 16 mg/m² had at least 20% improvement compared with 14% of the placebo group ($p < 0.001$); 57% of the patients receiving TNFR:Fc 16 mg/m² had at least 50% improvement compared with 7% of the placebo group ($p < 0.001$).[24]

Treatment was generally well tolerated. The most common adverse effect was redness at the injection site, with some patients experiencing mild discomfort. Injection site reactions were more common in the high-dose treatment group. There appeared to be an increased incidence of mild upper respiratory tract infections in patients receiving TNFR:Fc 2 and 16 mg/m². These conditions were not considered serious and no patient with upper respiratory tract symptoms discontinued the study. The contribution of TNF inhibition to these events needs further investigation and clarification. Early study discontinuations were mainly seen in the placebo (n = 21) and low-dose (n = 18) treatment groups, usually due to disease exacerbation. Other causes for study discontinuation were few and equally distributed across treatment groups. No anti-TNFR:Fc antibodies were detected during or after treatment with TNFR:Fc.[24]

Mean ESR, CRP, duration of morning stiffness, global assessment by patient and physician, HAQ, and VAS were improved in patients receiving the TNFR:Fc 2 and 16 mg/m² doses. The group receiving TNFR:Fc 16 mg/m² had a mean 31% decrease in ESR at weeks 4 and 12. The study was powered to determine the difference in dose-related efficacy and adverse effects during a short (3-mo) trial period.[24]

Conclusion

TNFR:Fc, a biologic TNF inhibitor, interferes with the cytokine cascade involved in the inflammatory process. In the Phase II clinical trial, TNFR:Fc was generally well tolerated and effective. TNFR:Fc is nonimmunogenic; no antibodies to TNFR:Fc have been detected thus far in human studies. Preliminary data indicate that TNFR:Fc is an excellent candidate for future long-term studies in the treatment of rheumatoid arthritis. ≃

References

1. Harris ED Jr. Clinical features of rheumatoid arthritis. In: Kelley WN, Harris ED Jr, Ruddy S, Sledge CB, eds. Textbook of rheumatology. 4th ed. Philadelphia: WB Saunders Co., 1993:874-9.
2. American College of Rheumatology Ad Hoc Committee on Clinical

Guidelines. Guidelines for the management of rheumatoid arthritis. Arthritis Rheum 1996;39:713-22.
3. Horton S, Rexman-Targoff BH, Thompson DF. Use of cyclosporine in rheumatoid arthritis. Ann Pharmacother 1993;27:44-6.
4. Gabriel SE, Jaakkimainen L, Bombardier C. Risk of serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs: a meta analysis. Ann Intern Med 1991;115:787-96.
5. Kirwan JR. The effect of glucocorticoids on joint destruction in rheumatoid arthritis. The Arthritis and Rheumatism Council Low-Dose Glucocorticoid Study Group. N Engl J Med 1995;333:142-6.
6. O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ, et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. N Engl J Med 1996;334:1287-91.
7. Willkens RF, Urowitz MB, Stablein DM, McDendry RJR Jr, Berger RG, Box JH, et al. Comparison of azathioprine, methotrexate, and the combination of both in the treatment of rheumatoid arthritis: a controlled clinical trial. Arthritis Rheum 1992;35:849-56.
8. Williams HJ, Ward JR, Reading JC, Brooks RH, Clegg DO, Skosey JL, et al. Comparison of auranofin, methotrexate, and the combination of both in the treatment of rheumatoid arthritis. Arthritis Rheum 1992;35: 259-69.
9. Old LJ. Tumor necrosis factor. Sci Am 1988;258(5):59-75.
10. David J, Terhorst C. Immune response mechanisms. In: Dale DC, Federman DD, eds. Scientific American medicine. New York: Scientific American Inc., 1995,VI:III;12,13.
11. Cope AP, Aderka D, Doherty M, Engelmann H, Gibbons D, Jones AC, et al. Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic diseases. Arthritis Rheum 1992;35:1160-9.
12. Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991;34:1125-32.
13. Butler DM, Feldmann M, Di Padova F, Brennan FM. p55 and p75 tumor necrosis factor receptors are expressed and mediate common functions in synovial fibroblasts and other fibroblasts. Eur Cytokine Netw 1994;5: 441-8.
14. Brennan FM, Maini RN, Feldmann M. TNF — a pivotal role in rheumatoid arthritis? Br J Rheumatol 1992;31:293-8.
15. Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W, Loetscher H. Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. Proc Natl Acad Sci U S A 1990;87:3127-31.
16. Roux-Lombard P, Punzi L, Hasler P, Bas S, Todesco S, Gallati H, et al. Soluble tumor necrosis factor receptors in human inflammatory synovial fluids. Arthritis Rheum 1993;36:485-9.
17. Krane SM, Harris ED. Rheumatoid arthritis. In: Dale DC, Federman DD, eds. Scientific American medicine. New York: Scientific American Inc., 1995,XV:II:2,3.
18. Data on file. Seattle, WA: Immunex Corporation, 1997.
19. Mohler KM, Torrance DS, Smith CA, Goodwin RG, Stremler KE, Fung VP, et al. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J Immunol 1993;151:1548-61.
20. Jacobs CA, Beckmann MP, Mohler K, Maliszewski CT, Fanslow WC, Lynch DH. Pharmacokinetic parameters and biodistribution of soluble cytokine receptors. Int Rev Exp Pathol 1993;34B:123-35.
21. Wooley PH, Dutcher J, Widmer MB, Gillis S. Influence of a recombinant human soluble tumor necrosis factor receptor Fc fusion protein on type II collagen-induced arthritis in mice. J Immunol 1993;151:6602-7.
22. Moreland LW, Margolies G, Heck LW, Sawey A, Blosch C, Hanna R, et al. Recombinant soluble tumor necrosis factor receptor (p80) fusion protein: toxicity and dose finding trial in refractory rheumatoid arthritis. J Rheumatol 1996;23:1849-55.
23. Moreland L. Multicenter Phase II study of recombinant human tumor necrosis factor receptor fusion protein (rho TNFR:Fc) in active rheumatoid arthritis. Presented at the Cytokines and Inflammation Meeting, Boston, MA, November 16, 1995.
24. Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997;337:141-9.
25. Felson DT, Anderson JJ, Boers M, Bombardier C, Chernoff M, Fried B, et al. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. Arthritis Rheum 1993;36:729-40.

EXTRACTO

OBJETIVO: El factor de necrosis de tumor (FNT) es el mediador más dominante en la cascada de citocinas, que causa inflamación y destrucción de coyunturas en artritis reumatoidea. Una nueva clase de agentes bajo investigación, los inhibidores biológicos de FNT, inhiben la actividad de FNT. La proteína humana de recombinación del receptor p75Fc (FNTR:Fc; Enbrel) bloquea la actividad de la citocina FNT. Los datos obtenidos durante estudios de fase I y II de FNTR:Fc en artritis reumatoidea serán repasados en este artículo.

MÉTODOS: Todos los datos disponibles sobre FNTR:Fc en el tratamiento de artritis reumatoidea fueron repasados. Estos datos incluyen la literatura publicada y datos en los archivos del manufacturero (Immunex).

RESULTADOS: FNTR:Fc ha adverso efectivo en muchos modelos de inflamación, incluyendo modelos de animales con artritis reumatoidea y en estudios clínicos de artritis reumatoidea. Las conclusiones de un estudio con ratones demostró que p75 FNTR es un antagonista natural de la inflamación mediada por FNT. Un estudio de fase I controlado por placebo, con dosis escalacias, evaluó la seguridad y eficacia de FNTR:Fc en pacientes con artritis reumatoidea. No se reportaron efectos adversos severos. Un estudio de fase II aleatorio, doble ciego, controlado por placebo, evaluó 180 pacientes con artritis reumatoidea activa a los cuales no respondían a tratamientos previos. Se observó una relación de dosis-respuesta en la cantidad de coyunturas sensibles e inflamadas, y los pacientes que recibieron la dosis más alta (16 mg/m²) de FNTR:Fc fueron los que más mejoraron. El tratamiento fue en general bien tolerado. El FNTR:Fc no es inmunogénico; hasta el momento no se han detectado anticuerpos en estudios humanos.

CONCLUSIONES: Los datos preliminares demuestran que FNTR:Fc es un candidato excelente para futuros estudios de uso prolongado en el tratamiento de artritis reumatoidea.

SONIA FELICO